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Discrepancies between ergosterol and the phospholipid fatty acid 18:2ω6,9 as biomarkers for fungi in boreal forest soils

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Abstract

Ergosterol and the phospholipid fatty acid (PLFA) 18:2ω6,9 are frequently used as fungal biomarkers in studies on soils, and in accordance with the ideal for biomarkers of microorganisms they are thought to turn over rapidly after cell death and lysis. These biomarkers should also show the same patterns and responses to perturbations of the studied system. Here, I report strong correlations, in natural boreal forests of contrasting fertility, between free ergosterol and PLFA 18:2ω6,9 ($r = 0.821$, $P = 0.007$, $n = 9$). Surprisingly, ergosterol, but not PLFA 18:2ω6,9, appears non-responsive to both large-scale tree girdling, which interrupts tree belowground C allocation to ectomycorrhizal fungi, and to long-term N-loading, which may have negative effects on both mycorrhizal and saprotrophic fungi. These results, therefore, question the use of ergosterol to monitor effects of soil perturbations on fungi in the field, but do not put into question the use of the biomarker in natural forest ecosystems.

Keywords: Boreal forests; Ergosterol; Fungal biomarkers; Mycorrhizal fungi; N fertilization; PLFA; Tree girdling

1. Introduction

The sterol ergosterol, ergosta-5,7,22-trien-3β-ol, and a phospholipid fatty acid (PLFA), 18:2ω6,9, are examples of membrane-bound molecules commonly used as fungal biomarkers in studies on soil (Ruzicka et al., 2000; Bååth and Anderson, 2003, and references therein). The membrane area is assumed to be well correlated with the bio-volume of microbial cells, and as all ideal biomarkers of microorganisms in soil they are thought to turn over rapidly after cell death and lysis (Tunlid and White, 1992). Ideally, different markers for the same microbial group should also show the same patterns and responses to perturbations of the studied system. That PLFA 18:2ω6,9 is a good fungal biomarker in soils was shown by Frostegård and Bååth (1996) when they found strong positive correlations ($r = 0.92$, $n = 11$) between the fungal specific ergosterol and PLFA 18:2ω6,9 in soils from cultivated fields, gardens, grasslands, and soils from beech and spruce forests. The two biomarkers were also strongly correlated in laboratory experiments studying composts consisting of mixtures of shredded straw and pig slurry, during which saprophytic fungal biomass increased over time (Klamer and Bååth, 2004). Another example of agreement between the two fungal bio-markers was found in laboratory incubations that aimed at reducing the abundance of mycorrhizal fungi in forest soils but not that of the saprophytic fungi (Bååth et al., 2004). Here, I tested (i) whether ergosterol and fungal PLFAs show the same patterns in undisturbed model forest ecosystems and (ii) whether they respond similarly to N-fertilization or tree girdling, a treatment which terminates the carbon supply to ectomycorrhizal fungi. I report large discrepancies between ergosterol and PLFA 18:2ω6,9 in their response to ecosystem perturbation in boreal forests, whereas in undisturbed boreal forest, the biomarkers were strongly correlated.

2. Materials and methods

2.1. Study sites

The natural boreal forest is a 130-y-old forest at Betsele northern Sweden (64°39′N, 18°30′E, 235 m altitude) with

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large variations in productivity, soil chemistry, and plant community composition (Giesler et al., 1998; Högberg, 2001; Högberg et al., 2003, 2006a). This forest was sampled along three transects, each encompassing a dwarf shrub (DS) forest type (C:N ratio 38.1±4.1, pH 4.0±0.2, 5.5±4.1 μg inorganic N g^{-1} organic matter (OM)), a short herb (SH) forest type (C:N ratio 22.9±1.9, pH 4.6±0.1, 5.9±2.2 μg inorganic N g^{-1} OM), and a tall herb (TH) forest type (C:N ratio 14.9±0.6, soil pH 5.3±0.1, 19.3±10.2 inorganic N g^{-1} OM). OM concentration (g OM g^{-1} dry soil) decreased from 83% in the DS forest type to 54% and 44% in the SH and TH forest types, respectively.

I also used a large-scale tree-girdling experiment (Högberg et al., 2001; Högberg and Högberg, 2002) at Åheden, northern Sweden (64°14′N, 19°46′E, 175 m altitude). This pine forest is classified as a DS forest type (C:N ratio 37.9±2.2, pH 3.7±0.0, 2.2±0.9 μg inorganic N g^{-1} OM) and was subjected to tree girdling early (EG) or late (LG) in the summer of 2000; there were three replicate plots for each of these treatments and the control (C) treatment. By girdling (cutting off the bark around the tree at breast–height), the direct flow of photosynthate C to roots and soil was terminated.

Lastly, I used a long-term N-loading experiment (Tamm, 1999; Högberg et al., 2006b) in a pine forest of DS forest type (C:N ratio 37.5±2.1, pH 4.1±0.1, 1.2±0.5 μg inorganic N g^{-1} OM) at Norrliden, northern Sweden (64°21′N, 19°45′E, 267 m altitude). Ammonium nitrate was applied annually to plots (30 × 30 m) at four rates, N0–N3, with three replicate plots per treatment. N0 is the untreated control, which receives only the background N deposition of ca. 3 kg ha^{-1} y^{-1}, while N1 received additionally ca. 34 kg N ha^{-1} y^{-1}, 1971–2004, and N2 twice the N dose of N1, while N3 received ca. 108 kg ha^{-1} y^{-1} 1971–1990, and is thus a high N treatment recovering from the previous high N load (Högberg et al., 2006b).

2.2. Soil sampling

Soil was sampled on 18, 25, and 26 August 2004 at Betsele, Norrliden, and Åheden, respectively. In each case, i.e., at a location along transects at Betsele or a plot in the experiments, three samples of the organic mor-layer (F + H horizons, approximately corresponding to Oe + Oa) were taken with a 0.15-m (diameter) corer and bulked together to represent one replicate. I quickly and carefully sorted out roots by passing the soil through a sieve (5 mm mesh size). A maximum time period of 3 h at 11–14 °C preceded storage on dry ice (−78 °C). Thereafter, samples were kept in a freezer (−20 °C). Sub-samples for standard chemical characterization of the soils were analysed fresh, as described below. At Betsele, sampling was done along the three separate transects ($N = 3$), each encompassing all three forest types. Thus, composited soil samples were collected in the DS forest type at three positions (at 0, 10, and 20 m distance, $n = 3$), similarly in the SH forest type (at 40, 50, 60 m distance) and in the TH forest type (at 80, 85, and 90 m distance). At Norrliden and Åheden, three plots of each treatment were sampled; the central 400 and 100 m^2 area of each plot was sampled randomly at Norrliden and Åheden, respectively. The mean values of three transects through each forest type (site Betsele) and the mean values of three plots of each treatment (sites Norrliden and Åheden) were used in the statistical analysis ($N = 3$).

2.3. Analysis

Soil pH was measured at a soil:water ratio of 1:3 (v v^{-1}). Dried soil samples (70 °C, 84 h) were analysed for C% and N% using a CN analyser coupled to a mass spectrometer (Ohlsson and Wallmark, 1999). Organic matter content of the mor-layer, extractable ammonium and nitrate were analysed as given in Högberg et al. (2006a). Free ergosterol was extracted from 10 mg root-free, freeze-dried and milled soil material. The sample was vortexed intensively together with 0.5 ml ethanol for 2 min, and then centrifuged at 11 400 g for 30 min. The extraction step was repeated twice and 50 μl of the resulting supernatant was injected and analysed in an HPLC system (Sundberg et al., 1999). The analysis was performed at the laboratory of Prof. Torgny Näsholm, Department of Forest Genetics and Plant Physiology, SLU, S-901 83 Umeå, Sweden. Analysis of fungal-specific PLFAs in soil (Tunlid and White, 1992; Frostegård and Bååth, 1996) followed the Bligh and Dyer (1959) method as modified by Frostegård et al. (1991, 1993). Lipids were extracted from frozen soil equivalent to 0.15 g OM and separated on silica gel columns (Bond Elut LRC, SI 100 mg, Varian, Palo Alto, CA, USA). Lipids were eluted in sequence with chloroform, acetone, and methanol. The chloroform fraction (containing neutral lipids, NLFAs) and the methanol fraction (containing PLFAs) were dried under N_2, dissolved and subjected to mild methanolysis. The resulting fatty acid methyl esters were analysed on a GC (PDZ Europa Ltd., Northwich, Chesire, England), equipped with a 25 m phenyl methyl siloxane column (internal diameter, 0.2 mm; film thickness, 0.33 μm) (Ultra 2 column, Agilent Technologies, Palo Alto, CA, USA). The identity of individual PLFA peaks were determined by comparing retention times of authentic FAME standards (FAME 37-47885-U, Supelco, Bellefonte, USA), and by comparing the retention times for individual PLFA peaks with peaks that had been identified by GC–MS. Methylnonadecanoic acid (Me19:0) was used as internal standard. Fatty acids are designated as the total number of C atoms:number of double bonds, followed by the positions of the double bonds. Two PLFAs were used as indicators of fungal biomass: 18:2ω6,9 (Federle, 1986; Frostegård and Bååth, 1996; Olsson, 1999) and 18:1ω9 (Bååth, 2003). The full data set on 18:2ω6,9 and 18:1ω9 and other PLFA biomarkers is reported in a study on the general microbial community structure at the same sites and experiments as studied here (Högberg M.N. et al.,